UNIVERSITI PUTRA MALAYSIA

DETECTION OF TRIGLYCERIDE BASED ON MICROWAVE DIELECTRIC PROPERTIES OF TRIGLYCERIDE ENZYMATIC REACTION

HASNIDAR BINTI HAMID
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By

HASNIDAR BINTI HAMID

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2010

DEDICATION

To my beloved mother and father whom I owe every success in my life

MARDIAH BINTI ISHAK
HAMID BIN HAMZAH

My dearest husband

MOHD KAMARULZAMAN BIN MANSOR

My little son

MUHAMMAD FATHI BIN MOHD KAMARULZAMAN

Siblings

My family in law

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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Chairman : Professor Kaida Khalid, PhD

Faculty : Science

Triglyceride is identified to have an important function in dairy products since it can cause milk rancidity and affect cheese flavor. In this study, the detection of triglyceride was done through the measurement of dielectric properties of triglyceride enzymatic reaction in the frequencies ranging from 200 MHz to 20 GHz by using Network Analyzer. This measurement is suitable to investigate the changes in dielectric properties of triglyceride and furthermore the glycerol and fatty acids formed from this reaction. The dielectric properties measured are dielectric constant, ε' and dielectric loss, ε". These dielectric properties are important in carrying out the investigation of molecules interaction of triglyceride as a substrate and lipase as a catalyst in the enzymatic reaction while irradiated with the electromagnetic field. This interaction can detect the

presence of ionic conductivity at low frequency and also dipole orientation at high frequency of microwaves frequency range. From the interaction of triglyceride with the presence of lipase as a catalyst, the expected products obtained are fatty acids and glycerol.

The glycerol and fatty acids formed from the reaction that can be seen as a cloudy upper layer liquid and a clear lower layer which can be observed after a stable condition at about 4 minutes after the reaction. By analyzing the dielectric properties of upper layer with the dielectric properties of the enzyme, a good relationship can be obtained between these values to the triglyceride concentration. For better sensitivity of the measurement, the chosen frequencies of the measurement are about 1 GHz, 2.6 GHz, 10 GHz and 15 GHz and the preferable mixing ratio between substrate and enzyme is 1:1. This condition is applied to the triglyceride and milks samples of concentration from 10000 ppm to 50000 ppm. The reading of dielectric properties was found to increase with the increasing concentration of the triglyceride and milk samples.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN TERHADAP TRIGLISERID BERDASARKAN CIRI-CIRI DIELEKTRIK PADA FREKUENSI GELOMBANG MIKRO TERHAPAD INTERAKSI TRIGLISERID DAN ENZIM

Oleh

HASNIDAR BINTI HAMID

Julai 2010

Pengerusi : Professor Kaida Khalid, Phd

Fakulti : Sains

Trigliserid telah dikenalpasti mempunyai fungsi yang cukup penting dalam produk tenusu dimana ia berfungsi mempengaruhi ketengitkan susu dan boleh mempengaruhi keenakan rasa keju. Maka dalam kajian ini, pengukuran ciri-ciri dielektrik terhadap interaksi trigliserid dan lipase telah dijalankan di dalam frekuensi gelombang mikro iaitu dari 200 MHz hingga 20 GHz dengan menggunakan Network Analyzer. Pengukuran ini dilihat sesuai untuk menyiasat dan menganalisa perubahan ciri-ciri dielektrik apabila trigliserid bertindakbalas dengan lipase serta mengkaji perubahan bacaan dielektrik terhadap produk yang terhasil. Ciri-ciri dielektrik yang diukur adalah pemalar dielektrik, ε’ dan kehilangan dielektrik, ε”. Kajian mengenai dielektrik ini

penting untuk memahami interaksi molekul-molekul pada frekuensi gelombang mikro. Interaksi trigliserid dan lipase ini adalah untuk melihat sejauh mana kehadiran spesies berion yang memberi kesan konduktif ion pada frekuensi yang lebih rendah atau kesan dwikutub yang menyebabkan kesan orientasi dwikutub pada frekuensi tinggi di mana-mana julat frekuensi gelombang mikro. Daripada interaksi ini juga, hasil yang dijangkakan akan terbentuk adalah asid-asid lemak dan gliserol.

Hasil daripada tindak balas berenzim ini mendapati lapisan atas yang terbentuk adalah larutan berwarna keruh manakala lapisan bawah adalah jernih. Keadaan ini dapat diperhatikan selepas 4 minit tindak balas berlaku. Dengan menganalisis perubahan nilai dielektrik bagi lapisan atas berbanding dengan nilai dielektrik trigliserid sebelum tindak balas, suatu hubungan baik telah dikenalpasti. Untuk mendapatkan sensitiviti pada keputusan yang diperolehi, frekuensi yang terbaik adalah pada 1 GHz, 2.6 GHz, 10 GHz dan 15 GHz dan nisbah tindak balas yang sesuai adalah 1:1. Hasil keputusan pada frekuensi dan nisbah diatas sesuai diaplikasikan pada julat kepekatan trigliserid dan susu antara kepekatan 10000 ppm hingga 50000 ppm dimana akan meningkat dengan kenaikan kepekatan trigliserid dan susu. Perkaitan yang positif juga ditunjukkan oleh trigliserid dan sampel susu. Perkaitan ini adalah bertepatan dengan mengaitkan perubahan ciri-ciri dielektrik pada kepekatan triglyserid dan susu.

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Last but not least, I would like to thank all the lecturers, staffs, friends and students in Department of Physics and Faculty of Science, Universiti Putra Malaysia for their help.

H. Hasnidar

I certify that a Thesis Examination Committee has met on 23th July 2010 to conduct the final examination of Hasnidar bt Hamid on her thesis entitled "Detection Of Triglyceride Based On Microwave Dielectric Properties Of Triglyceride Enzymatic Reaction" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Mohd Maarof H.A.Moksin, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Elias Saion, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Khamirul Amin Matori, PhD
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Nor Sabirin Mohamed, PhD
Associate Professor
Division of Physics
Faculty of Centre for Foundation Studies in Science
University Malaya
Malaysia
(External Examiner)

BUJANG BIN KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master. The members of the Supervisory Committee were as follows:

Kaida Khalid, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Nor Azah Yusof, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 August 2010

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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HASNIDAR BINTI HAMID

Date:

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LIST OF ABBREVIATIONS

TG	Triglyceride
NA	Network Analyzer
ppm	Part per million
GC	Gas Chromatography
TEM	Transverse Electromagnetic
OCP	Open-ended Coaxial Probe
ε'	Dielectric Constant
ε''	Dielectric Loss
ε'_{∞}	Dielectric constant at 'infinite' frequency
ε'_{s}	Static Dielectric constant
ε_0	Dielectric properties of free space
τ	Relaxation time
ω	Angular Frequency
f	Frequency
σ	Conductivity
P	Polarization
E	Electric Field Intensity
Γ	Reflection Coefficient
A_e	Empirical coefficient

CHAPTER 1

INTRODUCTION

1.1 Motivations of the Study

The investigation of dielectric behavior in microwave frequency range is opening more opportunity in many sensing area. The method potentially provides a low-cost, effective and rapid measurement. Moreover, microwave systems use a very low power levels and are non invasive techniques. Realizing this great importance of research within a microwave frequency range, a study of dielectric properties of Triglyceride (TG) – lipase enzymatic reaction was carried out to develop a TG biosensor. In this study, the interaction is between TG (substrate) and lipase (enzyme) to produce fatty acids and glycerol can be catalyzed by an enzyme as shown in Figure 1.1:

$$\text{Triglycerides} + 3\ H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + 3 \text{ molecules of fatty acids}$$

Figure 1.1: The enzymatic reaction of TG

Generally, TG can be found in animals' milk with a short chain of fatty acid. In milk fat, TG may affect cheese flavor and causes milk rancidity. The modulation of mouth feel of milks and dairy foods by TG may donate to the development of food choices and eating habits. With regard to TG as a main content in milks, the investigation on dielectric properties of TG is important in exploring the interaction of TG with electromagnetic fields. These include the study of TG interaction with lipase and the behavior of glycerol and fatty

acids. By measuring the dielectric properties of these enzymatic reaction in the microwaves interaction, two effects can be observed namely conduction loss which is due to ionic species or free electron at low microwave frequency region (below 2 GHz) and dipole orientation by dipole molecules at higher microwave frequency region (above 4 GHz).

Among the various methods available for TG determination, biosensors are comparatively more simple, sensitive, specific and rapid (Minakshi, 2008). The existence of biosensor in recent years becomes a powerful tool for the screening of a wide variety of compounds in all areas. One of the potential areas for an extensive usage of biosensor is in industries which is promising to give major advantages especially in food treatment. Realizing the needs of such advantage, the study of potentiometric biosensor for urea determination in milk has been done by Trivedi *et al*, (2009) who investigated an unbalanced feeding of cows that may influence the milk production and fertility. Moreover, the quality control and nutritional labeling of foods in the foodstuff industry is another biosensor application. For example, as reported by Shih *et al*, (2009), a biosensor was developed in order to determine the total cholesterol for the cardiac disease prevention by controlling and detecting the presence of cholesterol in food. For this reason, the study of biosensor on TG detection in milk gives a great significance in dairy industries and food processing technology. The principle of this TG detection is based on the analyzing of dielectric properties changes of fatty acids and glycerol that are the end product of TG - lipase enzymatic reaction.

The dielectric behavior of compounds has long been of interest, not only for the study of composition and structure, but also for food and scientific applications such as nutritional, food regulations, healthy assessment and consumer protection for public healthiness. The dielectric properties of most materials depend on the frequency of the applied alternating electric field, temperature, moisture content, density, composition and structure of the materials (Venkatesh and Raghavan, 2004). However, in this study, the measurement only depends on the frequency, composition and the concentration of the samples. It is possible to develop a TG biosensor for future research. Development on TG detection in milk industries should increase in order to monitor the quality of milk as well as its processing since these stages of manufacturing are depending on the TG content. This initiative is expected will help consumers to choose a better food for a healthy diet.

1.2 Scope of the Research

As the scope of this study, the measured values are the dielectric properties which investigate the dielectric constant, ε' and dielectric loss, ε". These properties will give the information about the trend of microwave interaction with TG and behavior of glycerol and fatty acids that formed from various parameters. The parameters are frequency, concentration of TG and milk samples and also the suitable ratio for reaction TG-lipase. The usage of coaxial probe is chosen since this method is non invasive and non destructive testing. But how great and convenience it is, the relation between the dielectric properties with the biological samples at microwave frequencies is a difficult task and not simple to understand and analyze. Structural studies of the samples were not

included due to the time limit. The investigation of the sample chemically and biologically is also not taking into account. This is because at microwave range, the dielectric properties of aqueous solutions or mixtures are decreased by two mechanisms which are the replacement of water by a substance and the binding of water molecules (Ryynänen, 1995). However, in this study, the focus of the measurement which are on their dielectric properties is not meant to be a definite example of how microwaves will be used to characterized the TG in milk, but in a hope as a indication of the form such might take, the data may look like, and what components of the TG enzymatic reaction may be easily detected.

1.3 Objectives of the Research

Referring to the brief idea explained in the beginning of this chapter, the purpose of this work is to investigate what happen in the enzymatic reaction of TG - lipase. This is due to the wide applications in sensing area by exploring the behavior of dielectric properties of bio samples in microwave frequency range. The investigation is an early work to apply the result of the proposed study in food industries for future needs. Working on this frequency range is mainly about the polarization of materials. Focusing on the dielectric behavior in foods provide a great extent which is useful in constructing ovens, in selecting packaging materials, and in developing foods (Ryynänen, 1995). The main objectives of the present work are as below:

1. To investigate the dielectric properties of glycerol and fatty acids formed from the TG - lipase enzymatic reaction at microwave frequencies (0.2 GHz - 20 GHz).
2. To correlate the dielectric properties of glycerol and fatty acids formed with suitable concentration of TG at specific frequency and several ratios.
3. To apply the above relationship for determination of concentration of TG in the milk samples such as full cream and low fat milk.

1.4 Brief Research Overview

This thesis is divided into six chapters. Chapter 1 is a general explanation about the idea and the focus of this research. This research is a beginning work of biosensor application within the microwaves frequency range for TG and milk samples.

In Chapter 2, a brief literature review is presented. It is a basic explanation of microwaves and previous study regarding to the effect of ionic and dipole. The applications of biosensor using TG are mentioned in this chapter. This chapter also focuses on the importance of TG detection. The basic theories of microwaves interaction with materials, dielectric properties, biosensor system and biological sample are discussed in Chapter 3. Chapter 4 provides samples preparation method for measurements and identification of biosensor interaction which is suitable for microwaves detection through dielectric measurement system. The experimental errors and also the calibration method were discussed in this chapter too.